PB85-214369

Neonatal and Maternal Body Burdens of Hexachlorobenzene (HCB) in Mice: Gestational Exposure and Lactational Transfer

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. REC PB85-214369
EPA/600/J-85/048		

4. TITLE AND SUBTITLE	5. REPORT DATE
NEONATAL AND MATERNAL BODY BURDENS OF HEXACHLOROBENZENE (HCB) IN MICE: GESTATIONAL EXPOSURE & LACTATIONAL TRANSFER (Journal Version)	1985
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
K.D. COURTNEY J.E. ANDREWS	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
EPA,HERL,ITD,TB, MD-82, RTP, NC 27711	ACAE1A
	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Fundamental and Applied Toxicology 5(1): 265-277, Feb 1985

16. ABSTRACT

Hexachlorobenzene (HCB), a ubiquitous lipophilic pollutant, was found to be readily transferred in the milk of lactating dams to their suckling neonates. Pregnant CD-1 mice were treated during gestation, and the body burdens of HCB in the neonates and the dams were determined during lactation. Also, neonates from dams treated with HCB during gestation were cross-fostered at birth to dams treated with corn oil during gestation. The body burdens of HCB were greater in the neonates exposed to HCB by lactational transfer than the neonates exposed only by gestational transfer. For a number of tissues, the body burden of HCB in the pups was similar for full litters and litters reduced to 2 pups per litter. The body burdens of the dams were greatly reduced during lactation with a loss of 95% of HCB. A similar reduction was seen in dams with litters reduced to 2 pups. Lactational transfer of HCB was a major route of excretion. Non-pregnant mice were studied concurrently. In contrast to the lactating mice, the non-pregnant mice had much higher tissue levels of HCB than the lactating mice. Other non-pregnant mice studied up to 63 days after treatment still had high tissue concentrations of HCB. This was in contrast to the lactating dams in which the HCB was nearly depleted during the 20 days of lactation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	14
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/J-85/048
JOURNAL ARTICLE

FUNDAMENTAL AND APPLIED TOXICOLOGY 5, 265–277 (1985)

Neonatal and Maternal Body Burdens of Hexachlorobenzene (HCB) in Mice: Gestational Exposure and Lactational Transfer[1]

K. DIANE COURTNEY AND JAMES E. ANDREWS

Toxicology Branch, Inhalation Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Neonatal and Maternal Body Burdens of Hexachlorobenzene (HCB) in Mice: Gestational Exposure and Lactational Transfer. COURTNEY, K. D., and ANDREWS, J. E. (1985). *Fundam. Appl. Toxicol.* **5**, 265–277. Hexachlorobenzene (HCB), a ubiquitous lipophilic pollutant, was readily transferred in the milk of lactating dams to their suckling neonates. Pregnant CD-1 mice were treated during gestation, and the body burdens of HCB in the neonates and the dams were determined during lactation. Also, neonates from dams treated with HCB during gestation were cross-fostered at birth to dams treated with corn oil during gestation. The body burdens of HCB were greater in the neonates exposed to HCB by lactational transfer than the neonates exposed only by gestational transfer. In many tissues, the concentration of HCB in the pups from full litters was similar to that in pups from litters reduced to two pups per litter. Lactational transfer of HCB from the dams to the pups was a major route of excretion in that 95% of HCB was depleted during 20 days of lactation. HCB depletion was similar in dams with whole litters, and those with litters reduced to two pups. © 1985 Society of Toxicology.

Hexachlorobenzene (HCB, perchlorobenzene, C_6Cl_6) is a fungicide which was registered for use on seed grains such as wheat, barley, oats, and rye. Technical grade material contains about 98% HCB and 1% PCNB (pentachloronitrobenzene). HCB also occurs as an industrial byproduct in the manufacture of perchloroethylene, chlorine, carbon tetrachloride, other chlorinated solvents, and pesticides and is used as a peptizing agent in the manufacture of nitroso rubber for tires. HCB is a global pollutant of the chlorinated hydrocarbon type that bioaccumulates and has a long half-life. HCB has been detected in human beings and many species of wildlife including mammals, birds, and fish. There have been a few episodes of overt toxicity in human beings from accidental exposure. These findings have been discussed in a review (Courtney, 1979).

Within the last decade most developed countries have added HCB to the list of compounds which are routinely monitored to detect environmental exposures and body burdens of chlorinated hydrocarbons (Eckenhausen *et al.*, 1981; Calabrese, 1982; Courtney, 1979). Of particular interest was the finding that HCB was detected in human breast milk in developed countries in which HCB is an industrial by-product. With the increased practice of breast-feeding babies in these countries, there has been concern with regards to the quality of breast milk. Placental and lactational transfer of HCB was documented in Turkey in the late 1950s when a large scale exposure to HCB occurred in a few towns when HCB-treated seed grain was used by the people to make bread instead of the intended agricultural use. The toxic effects of HCB were not as pronounced in adults as the toxicity seen in young children and infants. There was a 95% infant mortality

[1] Presented in part at the Society of Toxicology Meeting, 1981. This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

0272-0590/85 $3.00
Copyright © 1985 by the Society of Toxicology.
All rights of reproduction in any form reserved.

which virtually eliminated all children between the ages of 2 months and 5 years (Courtney, 1979; Peters *et al.*, 1966). Milk samples of some of the mothers had demonstrable HCB.

The current data which are being acquired in Turkey have shown that the problem of human contamination with HCB is a multigeneration problem. The infants that survived the toxicity in the 1950s are now adults and the women are breast-feeding their infants. HCB is detectable in milk samples from these women even though there has been no known HCB exposure since childhood (Peters *et al.*, 1978; Cripps *et al.*, 1980).

Laboratory studies have documented the placental transfer of HCB from exposure during gestation (Mendoza *et al.*, 1978; Courtney *et al.*, 1976, 1979) as well as the fetal exposure from maternal stores of HCB (Courtney and Andrews, 1979). However, no studies have compared body burdens resulting from placental transfer to those resulting from lactational transfer. Such information would be applicable to the contamination of human breast milk and is also necessary to better understand the results of the multigeneration animal studies which have become routine for many pesticides and industrial chemicals. Thus, this study was undertaken to determine the gestational and lactational transfer of HCB in mice and the resulting maternal and neonatal body burdens.

METHODS

Pregnant CD-1 mice with known insemination dates and nonpregnant CD-1 mice were purchased from Charles River Laboratories, Inc., Wilmington, Massachusetts. Detection of a vaginal plug denoted Day 0 of gestation. Mice were housed five per cage in polycarbonate cages containing heat-treated pine shavings (Betta Chips, North Eastern Products Inc., Warrensberg, N.Y.) and given feed (Wayne Lab Blox, Allied Mills Inc., Chicago, Ill.) and filtered water (automatic watering system) *ad libitum*. An ambient temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and a 12-hr light–dark cycle were provided. Cages were housed under spun polyester filter bonnets. A sentinel mouse program monitored the animal holding rooms for the presence of rodent viruses, bacteria, and other pathogens.

The average weight of the five mice per cage was used to calculate the dose of HCB for the mice in that cage. There was very little variation in the body weights. HCB dissolved in corn oil was administered by gastric intubation (ig) at a daily volume of 0.2 ml/mouse at dose levels of 0, 1, 10, 50, or 100 mg/kg depending on the experiment. Control mice received 0.2 ml corn oil daily. The HCB was obtained from the British Drug House Chemicals Ltd., Poole, England, and had a purity not less than 99.5%. The method for the extraction of tissue HCB into hexane and its quantification by gas liquid chromatography has been reported earlier (Courtney *et al.*, 1979). All tissue samples were weighed and frozen until assayed for HCB. Triglycerides were determined using the Pierce Triglyceride Kit, Curet and Company, Memphis, Tennessee.

A. Gestational/lactational transfer study. Pregnant CD-1 mice received 0, 10, or 50 mg HCB/kg body wt, ig, on Days 11–17 of gestation which are the last 7 days of gestation. Mice were allowed to deliver their pups. The pups were killed either on Day 1 postpartum (pp) or Day 20 pp. The dams were killed after nursing their pups on Day 19 or 20 pp due to the number of samples that could be processed on a day and these samples are identified in the tables as Day 20 pp mice. Some dams nursed whole litters while other dams had litters reduced to two neonates and these are referred to as "reduced litters." All mice within a treatment group, both control and treated, were killed on the same day.

Liver, brain, muscle, skin, fat, and blood were taken from Day 1 pp and 20 pp adult mice and assayed for HCB. Day 1 pp neonates were independently homogenized whole and individual aliquots taken for assay of HCB. Liver, brain, muscle, skin, suprascapular fat pad, perirenal fat, and whole blood were taken from the Day 20 pp weanlings and assayed for HCB.

B. Cross-fostering study. The cross fostering design is shown below.

	Fetal/neonatal exposure to HCB	
Group	Days of gestational exposure	Days of lactational exposure
A	0	0
B	6–16	0–4
C	6–16	0
D	0	0–4
E	0	3–4

Pregnant CD-1 mice received either 0, 50, or 100 mg HCB/kg body wt, ig, on Days 6–16 of gestation. Mice were allowed to deliver their pups normally. Half of each litter remained with their dam and half cross-fostered as follows: Control pups from mothers receiving corn oil throughout gestation either stayed with their mothers until Day 4 pp (Group A), or were cross-fostered

on Day 0 pp to mothers receiving HCB on Days 6–16 of gestation (Group D). Pups exposed by placental transfer to HCB on Days 6–16 of gestation either remained with their mothers until Day 4 pp (Group B), or were cross-fostered on Day 0 pp to dams receiving corn oil on Days 6–16 of gestation (Group C). Additionally, pups from corn oil-treated dams were cross-fostered on Days 3–4 pp (a 24-hr exposure period) to dams which had been treated with HCB on Days 6–16 of gestation (Group E). None of the above dams was treated during lactation and all of these dams nursed full litters. All dams and neonates were killed on Day 4 pp.

Liver, spleen, mammary gland, fat, and whole blood were taken from dams and assayed for HCB. The suprascapular fat pad, stomach with contents (milk), and the remaining carcass were taken from the Day 4 pp neonates and assayed for HCB concentration.

To determine the HCB body burden following a 24-hr lactational exposure, an additional group of nontreated pregnant CD-1 mice were allowed to deliver their pups and nurse them. On Day 3 pp these dams received either 50 or 100 mg HCB/kg body wt, ig, and continued nursing their young for 24 hr. On Day 4 pp, the dams and neonates were killed and tissues, taken from the dam and pups as described above, were assayed for HCB.

C. HCB depletion study. To compare the depletion of HCB body burdens in pregnant and nonpregnant

TABLE 1

GESTATIONAL/LACTATIONAL TRANSFER

	HCB dose (mg/kg)			
	0	1.0	10.0	50.0
	Day 1 postpartum			
No. dams	9	5	7	7
Body wt (g)	30.7 ± 1.0	30.0 ± 1.6	32.9 ± 1.3	31.3 ± 1.3
Liver/body wt %	6.1 ± 0.1	6.1 ± 0.2	6.5 ± 0.3	8.1 ± 0.6[a]
Pups born/litter	8.7 ± 0.6	5.8 ± 1.0[a]	10.3 ± 1.5	8.0 ± 1.8
Pup birth wt (g)	1.6 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.4 ± 0.1
Non pregnant mice				
No. mice	—	8	2	4
Body wt (g)	—	28.8 ± 0.6	31.4 ± 0.6	30.0 ± 2.5
Liver/body wt %	—	5.4 ± 0.1	4.8 ± 0.5	5.5 ± 0.4
	Day 20 postpartum			
Whole litter group				
No. dams	3	6	8	6
Body wt (g)	38.3 ± 1.1	38.3 ± 0.6	41.2 ± 2.0	37.8 ± 1.2
Liver/body wt %	8.0 ± 0.4	7.0 ± 0.2	6.9 ± 0.3	6.2 ± 0.4
Pups born/litter	7.0 ± 2.0	10.3 ± 0.6	12.0 ± 1.2	7.3 ± 2.0
Pup birth wt (g)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Reduced litter group				
No. dams	—	5	5	4
Body wt (g)	—	—	36.5 ± 0.7	36.5 ± 1.3
Liver/body wt %	—	—	5.5 ± 0.5	6.0 ± 0.4
Pups born/litter	—	9.4 ± 1.0	10.4 ± 1.2	13.0 ± 1.1
Pup birth wt (g)	—	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Nonpregnant mice				
No. dams		6	6	4
Body wt (g)	—	29.4 ± 1.6	32.8 ± 1.2	36.9 ± 2.2
Liver/body wt %	—	5.2 ± 0.2	4.9 ± 0.2	5.1 ± 0.2

Note. Toxicologic evaluation of CD-1 mice treated with HCB, ig, daily for the last 7 days of gestation and studied on Day 1 or 20 postpartum. Nonpregnant mice were studied concurrently. Values are means ± SE.

[a] $p < 0.05$ compared to control value.

TABLE 2

GESTATIONAL/LACTATIONAL DEPOSITION AND DEPLETION OF HEXACHLOROBENZENE (HCB)—MATERNAL VALUES

No. mice	Litter size	Kill day pp	Liver	Brain	Muscle	Skin	Fat	Whole blood
					1.0 mg/kg			
5	Whole	1	2.63 ± 1.88	2.10 ± 0.67	9.98 ± 7.65	3.90 ± 1.02	84.56 ± 18.68	4.55 ± 2.01
6	Whole	20	0.06 ± 0.06[a]	0.75 ± 0.60	—	0.11 ± 0.07[a]	0.47 ± 0.16[a]	—
(% Decrease Days 1–20)			(98.28 ± 1.72[b])	(64.20 ± 28.77)	—	(97.14 ± 1.40[c])	(99.44 ± 0.19[c])	—
5	Reduced	20	0.16 ± 0.16[d]	0.03 ± 0.03[d]	0.02 ± 0.02[d]	0.14 ± 0.05[d]	0.32 ± 0.12[d]	—
(% Decrease Days 1–20)			(94.00 ± 6.00)	(98.50 ± 1.40)	(99.80 ± 0.20)	(96.54 ± 1.39)	(99.68 ± 0.16)	—
8	Nonpreg	1	3.74 ± 2.06	5.70 ± 2.95	5.61 ± 1.91	4.53 ± 1.29	78.98 ± 40.30	2.29 ± 0.38
6	Nonpreg	20	0.74 ± 0.11[a,d]	0.53 ± 0.08[d]	1.42 ± 0.26[d]	3.42 ± 0.22[a,d]	11.97 ± 2.65[a,d]	—
(% Decrease Days 1–20)			(80.32 ± 2.94[b])	(90.63 ± 1.29)	(74.77 ± 4.25)	(24.57 ± 4.88[c])	(84.83 ± 3.34[c])	—
					10 mg/kg			
7	Whole	1	40.02 ± 18.20	9.08 ± 1.81	15.82 ± 4.60	60.77 ± 18.42	64.14 ± 26.09	15.94 ± 4.20[k]
8	Whole	20	0.21 ± 0.06[h]	0.24 ± 0.15[h]	0.35 ± 0.18[h]	0.18 ± 0.23[h]	1.51 ± 0.70[h]	0.26 ± 0.10[h]
(% Decrease Days 1–20)			(99.49 ± 0.15[i])	(96.11 ± 1.87)	(97.88 ± 1.13)	(94.86 ± 1.44[i])	(84.79 ± 12.54)	(98.41 ± 0.63[i])
5	Reduced	20	0.40 ± 0.12[m]	0.12 ± 0.04[m]	0.35 ± 0.21	0.60 ± 0.08[m]	1.00 ± 0.58[m]	0.71 ± 0.56[m]
(% Decrease Days 1–20)			(99.01 ± 0.31)	(98.68 ± 0.41)	(97.77 ± 1.31)	(99.02 ± 0.14[i])	(98.46 ± 0.90)	(95.55 ± 3.75)
2	Nonpreg	1	41.62 ± 1.11	—	—	—	—	38.24 ± 3.95[k]
6	Nonpreg	20	22.49 ± 4.39[h,m]	7.88 ± 1.53[h,m]	8.49 ± 4.10[h]	91.95 ± 17.11[h,m]	324.98 ± 115.05[h,m]	17.01 ± 3.30[h,m]
(% Decrease Days 1–20)			(42.64 ± 16.34[i])	—	—	—	—	(55.42 ± 12.75[i])
					50 mg/kg			
7	Whole	1	139.18 ± 47.85	106.24 ± 24.26	56.24 ± 4.04	208.91 ± 68.52	242.74 ± 91.75[t]	139.88 ± 23.15[t]
6	Whole	20	0.70 ± 0.13[s]	0.29 ± 0.08[s]	0.60 ± 0.30[s]	6.13 ± 3.15[s]	2.84 ± 1.56[s]	3.20 ± 2.56[s]
(% Decrease Days 1–20)			(99.49 ± 0.10[n])	(99.73 ± 0.08[n])	(98.94 ± 0.57)	(97.07 ± 1.51[n])	(98.83 ± 0.64[n])	(97.71 ± 4.49[n])
4	Reduced	20	1.14 ± 0.75[v]	0.30 ± 0.15[v]	0.32 ± 0.17	1.53 ± 0.57[v]	3.39 ± 2.48[v]	0.44 ± 0.11[v]
(% Decrease Days 1–20)			(99.18 ± 0.53)	(99.73 ± 1.20)	(99.43 ± 0.30)	(90.84 ± 0.44)	(98.61 ± 1.02)	(99.69 ± 0.08)
4	Nonpreg	1	123.43 ± 56.95	35.28 ± 28.98	193.98 ± 185.00	110.1 ± 59.0	3044.8 ± 906.5[t]	19.13 ± 10.37[t]
4	Nonpreg	20	34.64 ± 6.23[s,v]	16.68 ± 3.07[s,v]	10.67 ± 4.96[s]	388.9 ± 58.5[s,v]	3066.2 ± 167.7[s,v]	41.90 ± 1.47[s,v]
(% Change Days 1–20)			(−71.94 ± 5.05[n])	(−52.72 ± 8.71[n])	(−94.51 ± 2.56)	(+253.1 ± 51.1[n])	(+0.7 ± 3.51[n])	(+119.1 ± 7.7[n])

Note. HCB concentration in tissues of CD-1 mice treated with 0, 1, 10, or 50 mg/kg HCB, ig, on the last 7 days of gestation. Dams nursed either whole litters or litters which were reduced to two pups/litter on Day 1 postpartum. Nonpregnant CD-1 mice were studied concurrently. Mice were assayed Day 1 or 20 postpartum (pp). Values are means ± SE. Values with the same letter within columns, $p < 0.05$.

mice, additional nonpregnant CD-1 mice were treated ig for 11 consecutive days with 10, 50, or 100 mg HCB/kg body wt. Mice were killed on Day 1, 6, 14, 21, 28, 42, 63, or 120 after the last dose. Liver, skin, brain, muscle, fat, and whole blood were taken from each mouse and assayed for HCB and triglyceride content.

D. Statistical analysis. The data for all studies are presented as mean values with standard errors. The litter was considered the unit for calculations and statistics. Thus, the means were taken for each litter and then averaged across litters. Statistical evaluation was performed using ANOVA and Dunnett's multiple comparison procedure (Dunnett, 1955).

RESULTS

A. Gestational/Lactational Transfer of HCB

The gestational transfer of HCB as well as the gestational plus lactational transfer of HCB was determined by quantifying the HCB in Day 1 pp and Day 20 pp pups and their respective dams.

Adults. On Day 1 pp, there were no statistically significant differences in adult or pup body weight compared to the respective control values (Table 1). The maternal liver to body weight ratio was significantly increased in the mice receiving 50.0 mg HCB/kg. The number of pups born per litter was significantly less in the 1.0-mg HCB/kg dose group and significantly greater in the 10.0-mg HCB/kg dose group. The pup body weight in these two groups reflects the number of pups in the litter, and are probably not the result of the HCB exposure. On Day 20 pp, there were no significant differences in dam body weights, dam liver to body weight ratios, or number of pups born/litter of the HCB-treated mice compared to the values of the control mice. The liver to body weight value of the control mice was slightly higher than the value usually seen in control mice. The number of control pups in this group was small due to a large number of mice which were nonpregnant.

The values for the concentration of HCB are presented along with the percentage decrease in HCB concentration from Day 1 to Day 20 pp (Table 2). At the 1.0-, 10.0-, and 50.0-mg/kg dose levels on Day 1 pp, the values for the HCB concentration in the tissues of the dams with whole litters did not differ significantly from those in the tissues of the nonpregnant mice. The transfer of HCB from the tissues of the dams with whole littes and those with reduced litters was nearly complete by Day 20 postpartum. In contrast, the depletion of HCB from the tissues of the nonpregnant mice was significantly less than that from the tissues of the lactating mice, both those with whole litters and those with reduced litters.

Neonates. The average weights of the neonates of the 50-mg/kg dose group were slightly less than the litter average of the 1.0- and 10.0-mg/kg groups but this difference was not significant (Table 3). The concentration of HCB in the neonates was dose related. The HCB concentration in the neonates from the dams receiving 10 mg/kg was approxi-

TABLE 3

GESTATIONAL AND LACTATIONAL TRANSFER OF HEXACHLOROBENZENE (HCB)—NEONATAL VALUES

	(mg/kg)		
HCB dose	1.0	10.0	50.0
No litters	5	6	5
No. neonates assayed	19	23	20
Wt neonates assayed	1.7 ± 0.1	1.6 ± 0.1	1.4 ± 0.1
HCB–ppm/wet wt neonate	1.80 ± 0.16	9.63 ± 2.69	122.64 ± 33.12

Note. HCB concentration in Day 1 pp neonates of CD-1 mice treated with HCB, ig, daily for the last 7 days of gestation. Approximately four neonates/litter were assayed. Values are means ± SE.

TABLE 4

GESTATIONAL/LACTATIONAL TRANSFER OF HEXACHLOROBENZENE (HCB)—WEANLING VALUES

No. of litters	No. pups	Litter size	HCB (ppm)						
			Liver	Brain	Muscle	Skin	S-Fat[a]	P-Fat[b]	Blood
					1.0 mg/kg				
6	24	Whole	0.40 ± 0.6	0.28 ± 0.05	0.71 ± 0.22[c]	1.23 ± 0.19[d]	2.07 ± 0.89[e]	4.18 ± 1.4	—
5	10	Reduced	0.52 ± 0.12	0.29 ± 0.05	1.77 ± 0.33[c]	2.94 ± 0.61[d]	8.23 ± 1.83[e]	4.06 ± 1.99	—
					10.0 mg/kg				
8	32	Whole	7.19 ± 1.80	3.75 ± 0.92	10.82 ± 3.73	48.15 ± 19.49	66.99 ± 14.47	85.17 ± 10.51	10.33 ± 4.42
5	10	Reduced	6.91 ± 0.09	2.76 ± 0.50	14.24 ± 2.10	56.85 ± 8.12	104.56 ± 9.90	289.50 ± 31.91	3.92 ± 0.81
					50.0 mg/kg				
6	22	Whole	27.9 ± 5.6	16.7 ± 3.9	32.7 ± 8.3	122.8 ± 30.8	299.5 ± 99.4	191.5 ± 119.6	26.8 ± 4.3
4	9	Reduced	22.3 ± 3.6	11.3 ± 4.1	45.0 ± 28.0	186.3 ± 50.6	249.1 ± 38.8	521.7 ± 103.5	14.3 ± 3.7

Note. Concentration of HCB in tissues of 20-day-old pups of CD-1 mice treated with 1.0, 10.0, or 50.0 mg/kg HCB, ig, during the last 7 days of gestation. The mice nursed whole litters or litters which were reduced to two pups/litter on Day 1 postpartum. Values are means ± SE. Values with the same letter were significantly different with $p < 0.05$.

[a] Suprascapular fat pad.

[b] Perirenal fat.

mately fivefold that found in the neonates from dams receiving 1 mg/kg. However, the concentration of HCB in the neonates from the dams receiving 50 mg/kg was slightly more than 10 times that seen in the neonates from the dams receiving 10 mg/kg.

The concentration of HCB in the tissues of the 20-day-old pups is presented in Table 4. Approximately four pups per litter were randomly chosen and independently assayed for the HCB concentration in their tissues. Each of the two pups from the reduced litters were assayed individually for HCB content. In the 1.0-, 10.0-, and 50.0-mg/kg dose groups, there were no statistically significant differences in the concentration of HCB between whole and reduced litters for most tissues. The HCB concentration in the pups was dose related, and the highest concentrations of HCB were found in the tissues with the highest fat content such as the suprascapular fat pad, perirenal fat, and skin.

B. Cross-Fostering Study

The body weights, liver weights, and liver to body weight ratios of the dams treated with 50 mg HCB/kg were significantly different from the corresponding values seen in the corn oil-treated dams, while the spleen weight, spleen to body weight ratio, and number of neonates per litter in the two groups were not significantly different from corresponding values in the control dams (Table 5). The control dams had detectable and quantifiable HCB in their tissues since they nursed neonates which were cross-fostered from the HCB-treated dams. By caring for these neonates, the control dams were exposed to HCB. The rank order of the tissue concentrations of HCB was the same in the control and treated dams.

The control pups of the cross-fostering study had detectable HCB resulting from contamination by the HCB exposed pups in the same litter (Table 6). In pups, the fat sample was taken from the suprascapular fat pad since perirenal fat had not developed to any extent at this age. The stomachs containing the milk were removed and analyzed separately since the amount of milk and the concentration of HCB in the milk can be a variable parameter. The body weight of the pups exposed to HCB throughout gestation and lactation, and the pups exposed only during lactation were significantly ($p < 0.01$) reduced compared to the respective values for the control pups. The concentration of HCB in the tissues and the whole pup body burden were the highest in the pups exposed during both gestation and lactation, followed by the pups exposed only during lactation.

Lactational transfer of HCB for a 24-hr period was estimated by cross-fostering some control pups on Day 3 pp to HCB dams which had been treated on Days 6–16 of gestation and allowed to nurse their litters until Day 3 pp. Pups from control litters

TABLE 5

CROSS-FOSTERING—MATERNAL VALUES

	Maternal values	
	Corn oil	HCB
Dose	0[a]	50 mg/kg
No. dams	13	7
Body wt (g)	38.0 ± 0.8	35.2 ± 0.8[b]
Liver wt (g)	2.2 ± 0.1	2.7 ± 0.1[c]
Liver/body × 100	6.0 ± 0.1	8.0 ± 0.6[d]
Spleen wt (g)	172.7 ± 8.4	145.0 ± 8.2
Spleen/body × 100	0.46 ± 0.03	0.36 ± 0.04
Neonates/litter	10.8 ± 0.3	9.9 ± 1.1
	HCB (ppm)	
Spleen	0.4 ± 0.2	46.0 ± 23.7
Liver	0.9 ± 0.4	83.8 ± 41.9
Whole blood	1.2 ± 0.3	688.9 ± 565.1
Mammary gland	3.2 ± 1.3	911.7 ± 388.5
Perirenal fat	8.8 ± 2.4	2029.2 ± 948.0

Note. Hexachlorobenzene (HCB) concentrations and toxicological data from CD-1 mice treated with 50 or 100 mg/kg HCB, ig, on Days 6–16 of gestation and killed on Day 4 postpartum. Values are means ± SE.

[a] 0.2 ml/mouse.

[b] $p < 0.05$ compared to the corn oil group.

[c] $p < 0.05$ compared to the corn oil group.

[d] $p < 0.05$ compared to the corn oil group.

TABLE 6

CROSS-FOSTERING—NEONATAL VALUES

No. dams	No. pups	Days of pup HCB exposure: Gest.[a]	Days of pup HCB exposure: pp	Neonate body wt (g)—Day 4	HCB (ppm): Suprascapular fat	HCB (ppm): Stomach (milk)	HCB (ppm): Carcass	μg HCB/neonate
Corn oil control dams								
12	50	None	None	2.6 ± 0.2	6.7 ± 4.2	4.9 ± 2.8	1.7 ± 0.8	3.9 ± 1.6
50 mg/kg HCB-treated dams								
4	9	6–16	0–4	1.7 ± 0.2[b]	392.8 ± 117.0	479.1 ± 228.9	395.1 ± 94.3	577.9 ± 105.7
9	21	6–16	None	2.5 ± 0.1	306.1 ± 71.5	12.5 ± 3.6	31.4 ± 8.1	70.3 ± 16.4
3	6	None	0–4	1.6 ± 0.1[b]	391.1 ± 107.5	136.2 ± 19.6	258.3 ± 36.1	413.0 ± 68.2
3	11	None	3–4	2.5 ± 0.1	63.4 ± 31.6	83.0 ± 29.8	11.0 ± 7.2	29.8 ± 15.3

Note. Hexachlorobenzene (HCB) concentrations in CD-1 mouse pups on Postpartum Day 4 after exposure during gestation, lactation, or gestation and lactation to HCB-treated dams treated with 50 mg/kg, ig, on Days 6–16 of gestation. Values are means ± SE.

[a] Gestation.

[b] $p < 0.01$ compared to control value.

were exchanged for pups in the treated litter. Low values for HCB concentration obtained by this method did not reflect the expected values obtained from pups which were exposed during the 4 pp days. Thus, additional mice were studied by treating nonexposed lactating dams on Day 3 pp with HCB and assaying their pups on Day 4 pp (Table 7). There were no statistically significant differences in the maternal or neonatal body or organ weights between the 50- and the 100-mg/kg dose groups. There were no overt signs of toxicity in these dams treated with 100 mg HCB/kg. The concentration of HCB in the tissues of the dams at the 100-mg/kg dose level was approximately twice that in the tissues of the dams at the 50-mg/kg dose level. Thus, the deposition in the dams was dose related. The HCB concentration in the neonatal tissue of the 100-mg/kg dose group was only 1.5 times as high as that of the 50-mg/kg dose group; however, this difference was statistically significant. The concentration of HCB in the neonates was in keeping with the values obtained in the cross-fostering study.

C. HCB Depletion in the Mouse

All the mice gained weight between the beginning and end of the study (Table 8). There were no statistically significant differences in the body or liver weights between the three dose groups. The HCB concentration in the blood and fat showed a dose-related decrease in the concentration of HCB with time.

Dose–time-related decreases in the concentrations of HCB in the liver, skin, brain, and muscle also were evident (Table 9). Since laboratory mice tend to accumulate fat as they age, and since HCB is lipophilic, the fat content of these tissues was also determined. Generally, the triglyceride concentration remained fairly stable in these tissues over the time period of this study while the HCB concentration steadily decreased. Of partic-

TABLE 7

LACTATIONAL TRANSFER—SINGLE DOSE, 24 hr

	HCB dose (mg/kg)	
	50	100
No. dams	4	3
Body wt (g)	42.30 ± 1.10	41.30 ± 0.76
Liver/body × 100	6.37 ± 0.09	6.55 ± 0.13
Spleen/body × 100	0.46 ± 0.04	0.43 ± 0.09
No. in litter	9.25 ± 0.85	11.10 ± 1.00
No. neonates assayed	37	33
Neonate wt (g)	2.51 ± 0.16	2.75 ± 0.34
Carcass wt (g)	2.42 ± 0.17	2.68 ± 0.33
Stomach wt (g) (milk)	0.09 ± 0.02	0.07 ± 0.01
	HCB (ppm)	
Maternal tissue		
Whole blood	11.87 ± 1.79	20.73 ± 6.68
Liver	10.17 ± 3.54	16.44 ± 1.38
Spleen	4.25 ± 0.81	6.78 ± 0.94
Fat	258.77 ± 68.83	421.75 ± 11.99
Mammary gland	17.20 ± 6.12	54.67 ± 42.51
Neonatal tissue		
Stomach (milk)	132.96 ± 14.89	166.43 ± 13.62
Carcass	31.85 ± 1.17	47.30 ± 6.23*
	μg/neonate	
Neonate	89.72 ± 7.94	127.11 ± 4.14*

Note. Hexachlorobenzene (HCB) concentrations in CD-1 mice and neonates on Postpartum Day 4 resulting from dams receiving 50 or 100 mg/kg HCB, ig. on Postpartum Day 3 and killed on Day 4 pp. Values are means ± SE.

* $p < 0.05$, 50 vs 100 mg/kg.

ular interest is the apparent nonlinear depletion in the different tissues. This occurred on Days 21–28 for liver and skin, and on Days 14–21 for blood, fat, and brain, at the 10.0-mg/kg dose level. At the 50-mg/kg dose level, it occurred on Days 14–21 for blood, and Days 28–42 for liver, skin, brain, and muscle. This apparent plateau in HCB concentration may be due to an artifact or redistribution of HCB. The leveling off of the concentration in the fat on Days 14–21, with less marked leveling off on Days 28–42, suggests that the elimination of HCB from fat may not follow first order kinetics. In any case, HCB was slowly eliminated from the tissues and the depletion was dose–time related. This depletion over many weeks was in marked contrast to the depletion of HCB from the lactating mouse where depletion was nearly complete in about 20 days.

DISCUSSION

The transfer of HCB by the placenta and mammary gland is expected since many of the chlorinated hydrocarbons are transferred to the fetus and neonate. However, HCB differs from other chlorinated hydrocarbons such as DDT and PCBs in that HCB is not manufactured intentionally as the other compounds were, and therefore, the manufacturing of HCB cannot be stopped or curtailed as readily. In the EPA report (EPA, 1975) on HCB, it was estimated that only 15,000 lb/year of HCB were manufactured for fungicidal use while 250,000 lb/year were produced as a contaminant in the pesticide Dacthal and 1,400,000 lb/year were produced as a contaminant in the pesticide PCNB (pentachloronitrobenzene). It was estimated that 4,300,000 lb/year of HCB were produced as an unwanted byproduct. HCB is present in the wastes from some of these processes at levels of 10–15%. These waste products are referred to as "hex" wastes, and methods for their safe disposal are being developed (Quinlivan and Chassemi, 1977). HCB is volatile and sublimes at the air/soil interface contaminating areas around manufacturing sites, disposal sites, laboratories, and animal rooms (EPA, 1973; Laska *et al.*, 1976; IARC, 1979). However, HCB is very persistent in soil at depths greater than 2 cm (Beall, 1976; Isensee *et al.*, 1976). Although HCB has limited water solubility, it has been recognized as one of the major marine water pollutants (Morse, 1975).

HCB is slowly eliminated from body depots in the mouse as shown in this report as well as in sheep (Mull *et al.*, 1978), cows (Dingle and Palmer, 1977), rats (Villeneuve *et al.*, 1974), and humans (Peters *et al.*, 1966, 1978; Cripps *et al.*, 1980). Morita and Oishi (1975) suggested that the clearance from rats occurred in two steps: a fairly rapid process for

TABLE 8

DEPLETION OF HEXACHLOROBENZENE (HCB) CONCENTRATION—NONPREGNANT MICE

Mice	Days postdose	Body wt (g)	Liver wt (g)	HCB concentration (ppm)	
				Blood	Fat
			10 mg/kg		
6	1	33.4 ± 1.5	1.74 ± 0.09	78.1 ± 16.7	2953 ± 191
5	6	33.0 ± 0.5	1.54 ± 0.08	78.3 ± 20.6	1740 ± 122
5	14	32.3 ± 0.4	1.38 ± 0.03	26.5 ± 2.3	1062 ± 134
5	21	35.2 ± 0.6	1.87 ± 0.08	19.7 ± 6.1	1074 ± 150
5	28	36.9 ± 0.7	1.86 ± 0.10	8.9 ± 1.7	882 ± 141
5	42	36.9 ± 1.5	1.91 ± 0.14	3.9 ± 0.4	206 ± 30
5	63	40.4 ± 1.7	1.88 ± 0.06	4.3 ± 0.8	150 ± 39
			50 mg/kg		
6	1	34.6 ± 1.0	2.11 ± 0.21	259.3 ± 87.6	5801 ± 750
5	6	35.1 ± 1.2	2.18 ± 0.23	177.8 ± 26.1	3718 ± 389
5	14	36.3 ± 0.9	2.21 ± 0.13	91.4 ± 12.7	2130 ± 252
5	21	35.6 ± 0.9	2.02 ± 0.09	128.6 ± 16.7	2038 ± 219
5	28	38.2 ± 0.6	2.22 ± 0.06	40.7 ± 4.7	1328 ± 99
6	42	35.7 ± 1.1	1.94 ± 0.32	25.8 ± 3.7	1049 ± 187
5	63	39.2 ± 1.2	1.83 ± 0.06	21.3 ± 5.4	603 ± 79
7	120	36.6 ± 1.4	1.51 ± 0.07	2.0 ± 0.3	86 ± 7
			100 mg/kg		
6	120	46.5 ± 4.0	1.77 ± 0.09	3.7 ± 0.8	176 ± 12

Note. HCB concentration in blood and fat of nonpregnant adult female CD-1 mice treated ig with 10, 50 or 100 mg/kg HCB for 11 days and assayed 1, 6, 14, 21, 28, 42, 63, or 120 days after last dose. Values are means ± SE.

the first 14 days and then a much slower elimination for the next 56 days. The depletion of HCB in the nonpregnant mice in this report also appeared as a biphasic decline in concentration, with the break between Days 14 and 28 depending on the tissue.

The rate of depletion of HCB in the lactating mouse was considerably faster than that for the nonpregnant mouse. Villeneuve (1975) and Villeneuve *et al.* (1977) reported that food restriction resulted in mobilization of HCB from the fat depots to other tissues in rats; however, fecal analysis indicated that food restriction did not increase the excretion of HCB. HCB is excreted mainly unchanged in the feces in mice (Courtney *et al.*, 1976), as well as rats and monkeys (Rozman *et al.*, 1975). The few metabolites excreted in the urine are pentachlorophenol, pentachlorothiophenol, and tetrachlorohydroquinone (Koss *et al.*, 1976, 1978; Mehendale and Matthews, 1973; Brandt *et al.*, 1980; Richter *et al.*, 1981). Some of the metabolites may be carcinogenic or promoters of cancers as shown by Stonard (1975), Stonard and Nenov (1974), and Shirai *et al.* (1978).

Another route of elimination of HCB and metabolites is biliary excretion. Ingebrigtsen *et al.* (1981) have reported that 2.0 and 1.8% of the ^{14}C-HCB dose were excreted in the 24-hr bile sample as unchanged HCB and pentachlorobenzene, respectively. Sulfur-containing metabolites were not identified but hepatic glutathione decreased by 50%

TABLE 9

DEPLETION OF HEXACHLOROBENZENE (HCB) CONCENTRATION IN TISSUES—NONPREGNANT MICE

No. mice	Days postdose	Liver		Skin		Brain		Muscle	
		TRG[a]	HCB[b]	TRG	HCB	TRG	HCB	TRG	HCB
					10 mg/kg				
6	1	2.3 ± 0.9	105.9 ± 23.0	63.7 ± 21.2	271.6 ± 59.9	3.5 ± 0.7	99.6 ± 43.3	4.0 ± 1.0	24.3 ± 5.3
5	6	1.8 ± 0.9	114.7 ± 19.4	—	—	—	—	—	—
5	14	0.4 ± 0.1	33.7 ± 3.0	73.8 ± 35.2	176.0 ± 10.6	6.7 ± 0.8	10.9 ± 1.5	11.6 ± 4.7	60.1 ± 9.2
5	21	2.2 ± 1.3	22.5 ± 4.8	92.5 ± 22.1	121.0 ± 25.3	4.5 ± 0.1	25.3 ± 9.0	14.8 ± 4.0	34.2 ± 7.8
5	28	2.0 ± 0.6	19.5 ± 2.0	61.6 ± 19.0	273.9 ± 47.2	5.7 ± 1.7	6.5 ± 1.0	14.2 ± 2.3	8.7 ± 0.6
5	42	1.9 ± 0.5	9.2 ± 1.5	77.5 ± 30.9	51.0 ± 13.6	6.1 ± 1.6	4.7 ± 1.7	20.8 ± 5.4	17.0 ± 6.6
4	63	1.5 ± 0.4	4.1 ± 0.6	98.6 ± 28.3	65.7 ± 6.3	3.5 ± 0.5	1.5 ± 0.4	14.9 ± 3.8	7.9 ± 1.6
					50 mg/kg				
6	1	1.7 ± 1.0	249.5 ± 12.9	50.7 ± 23.1	1553.8 ± 166.1	4.4 ± 1.3	127.9 ± 28.5	7.2 ± 2.5	103.1 ± 25.6
5	6	1.3 ± 0.4	238.0 ± 76.2	—	—	—	—	—	—
5	14	0.5 ± 0.2	80.7 ± 23.9	45.9 ± 14.1	354.3 ± 81.3	7.9 ± 1.3	32.8 ± 6.3	12.4 ± 6.0	60.4 ± 12.8
5	21	1.2 ± 0.4	78.1 ± 19.8	124.4 ± 27.0	600.6 ± 84.3	6.0 ± 1.0	61.7 ± 16.8	23.4 ± 10.0	121.9 ± 3.2
5	28	1.8 ± 0.5	47.9 ± 13.0	98.5 ± 23.9	358.6 ± 77.6	5.3 ± 1.5	18.1 ± 2.3	8.2 ± 2.3	34.8 ± 9.7
6	42	0.7 ± 0.1	63.5 ± 10.3	123.0 ± 25.5	329.9 ± 20.5	5.4 ± 0.7	34.0 ± 7.3	33.9 ± 11.4	88.7 ± 16.2
5	73	1.5 ± 0.4	17.0 ± 3.1	86.8 ± 34.6	250.3 ± 44.4	5.8 ± 0.9	24.1 ± 15.1	23.2 ± 5.9	39.0 ± 6.5
7	120	2.4 ± 0.8	2.5 ± 0.5	130.7 ± 34.3	27.1 ± 5.4	6.9 ± 1.9	0.9 ± 0.2	32.8 ± 9.7	7.4 ± 3.5
					100 mg/kg				
6	120	1.6 ± 0.7	3.9 ± 1.4	86.6 ± 39.4	25.9 ± 4.7	4.3 ± 0.6	1.7 ± 0.4	45.2 ± 32.0	6.9 ± 2.8

Note. HCB and triglyceride (TRG) concentrations in tissues of nonpregnant CD-1 adult female mice treated IG with 10, 50, or 100 mg/kg HCB for 11 consecutive days and assayed 1, 6, 14, 21, 28, 42, 63, or 120 days after last dose. Values are means ± SE.

[a] TRG—triglycerides, mg/gm wet wt of tissue.

[b] HCB, ppm/wet wt tissue.

within the 24 hr after HCB treatment. Klaassen and Strom (1978) reported that the rate of bile production was increased by 50% in the lactating rat compared to adult males and nonlactating females. The bile production increased during pregnancy but decreased rapidly in the dam upon removal of the pups. The increased bile production was of canalicular origin and was due to an increase in both the bile salt-dependent and independent fractions with the lactating rats excreting some xenobiotics more rapidly than nonpregnant rats. Biliary excretion of HCB was not measured in the present study but could very well account for part of the increased elimination of HCB from pregnant and lactating mice, especially those with reduced litters.

HCB is not the only chlorinated hydrocarbon found in breast milk. Other chlorinated hydrocarbons such as DDT and PCBs are just as prevalent and some of the monitoring data show that the calculated intake of DDT in breast milk exceeds the acceptable daily intake (ADI) set by FAO/WHO (Hofvander *et al.*, 1981). HCB is now a ubiquitous pollutant. With the increase in breast feeding (Machol, 1980), and with the passing of HCB from mother to offspring, it may take a few generations to decrease body burdens assuming that no additional HCB enters the environment.

Neonates exposed by lactational transfer of HCB on Days 3 to 4 pp had a body burden of approximately 90 μg of HCB per neonate. Since this 90 μg value is comparable to the 417 μg of HCB per neonate which accumulated over 0 to 4 days pp, it was estimated that about 90 μg of HCB per neonate per day were transferred from dam to neonate at the 50-mg/kg dose level. At the 100-mg/kg dose level, approximately 130 μg of HCB were transferred to the neonate in the 24-hr period from Days 3 to 4 pp. At both dose levels, HCB readily accumulated in the neonate while the maternal body burden was reduced.

ACKNOWLEDGMENTS

The authors thank John Dew and Shusmita De for the excellent technical assistance and Joanne Cook for typing this manuscript.

REFERENCES

BEALL, M. L., JR. (1976). Persistence of aerially applied hexachlorobenzene on grass and soil. *J. Environ. Qual.* **5,** 367–369.

BRANDT, I., LYDEN, A., SLANINA, P., AND ALBANUS, L. (1980). Hexachlorobenzene (HCB) and some of its sulfur-containing metabolites: Tissue retention and excretion routes in mice. *Dev. Toxicol. Environ. Sci.* **8,** 627–630.

CALABRESE, E. J. (1982). Human breast milk contamination in the United States and Canada by chlorinated hydrocarbons, insecticides and industrial pollutants: Current status. *J. Amer. Coll. Toxicol.* **1,** 91–98.

COURTNEY, K. D. (1979). Hexachlorobenzene (HCB): A review. *Environ. Res.* **20,** 225–266.

COURTNEY, K. D., AND ANDREWS, J. E. (1979). Mobilization of HCB during gestation. *Toxicol. Lett.* **3,** 357–361.

COURTNEY, K. D., ANDREWS, J. E., AND SVENDSGAARD, D. J. (1979). Hexachlorobenzene (HCB) deposition in maternal and fetal tissues of mouse and rat. I. Chemical quantification of HCB in tissues. *Environ. Res.* **19,** 1–13.

COURTNEY, K. D., COPELAND, M. F., AND ROBBINS, A. (1976). The effects of pentachloronitrobenzene, hexachlorobenzene and related compounds on fetal development. *Toxicol. Appl. Pharmacol.* **35,** 239–256.

CRIPPS, D. J., GOCMEN, A., AND PETERS, H. A. (1980). Porphyria Turcica twenty years after hexachlorobenzene intoxication. *Arch. Dermatol.* **116,** 46–50.

DINGLE, J. H. P., AND PALMER, W. A. (1977). Residues of hexachlorobenzene in subcutaneous and butter fat of cattle. *Aust. J. Exp. Agric. Anim. Husb.* **17,** 712–717.

DUNNETT, C. W. (1955). A multiple comparison procedure. *J. Amer. Stat. Assoc.* **50,** 1096–1121.

ECKENHAUSEN, F. W., BENNETT, D., BEYNON, K. I., AND ELGAR, K. E. (1981). Organochlorine pesticide concentrations in perinatal samples from mothers and babies. *Arch. Environ. Health* **36,** 81–92.

EPA (1973). *Environmental Contamination from Hexachlorobenzene.* Ofice of Toxic Substances, Environmental Protection Agency, Washington, D.C.

EPA (1975). *Report on HCB.* Office of Toxic Substances, Environmental Protection Agency, Washington, D.C.

HOFVANDER, Y., HAGMAN, U., LINDER, C. E., VAZ, R., AND SLORACH, S. A. (1981). WHO collaborative

breast feeding study. I. Organochlorine contaminants in individual samples of Swedish human milk, 1978–1979. *Acta Paediatr. Scand.* **70,** 3–8.

IARC (1979). *Monograph of the Carcinogenic Risk of Chemicals to Man. Vol.* 20, pp. 155–178. IARC, Lyon.

INGEBRIGTSEN, K., SKAARE, J. U., NAFSTAD, I., AND FORDE, M. (1981). Studies on the biliary excretion and metabolites of hexachlorobenzene in the rat. *Xenobiotica* **11,** 795–800.

ISENSEE, A. R., HOLDEN, E. R., WOOLSON, E. A., AND JONES, G. E. (1976). Soil persistence and aquatic bioaccumulation potential of hexachlorobenzene (HCB). *J. Agric. Food Chem.* **24,** 1210–1214.

KLAASSEN, C. D., AND STROM, S. C. (1978). Comparison of biliary excretory function and bile composition in male, female and lactating female rats. *Drug Metab. Distrib.* **6,** 120–124.

KOSS, G., KORANSKY, W., AND STEINBACH, K. (1976). Studies on the toxicology of hexachlorobenzene. II. Identification and determination of metabolites. *Arch. Toxicol.* **35,** 107–114.

KOSS, G., SEUBERT, S., SEUBERT, A., KORANSKY, W., AND IPPEN, H. (1978). Studies on the toxicology of hexachlorobenzene. III. Observations in a long term experiment. *Arch. Toxicol.* **40,** 285–294.

LASKA, A. L., BARTELL, C. K., AND LASETER, J. L. (1976). Distribution of hexachlorobenzene and hexachlorobutadiene in water, soil, and selected aquatic organisms along the Lower Mississippi River, Louisiana. *Bull. Environ. Contam. Toxicol.* **15,** 535–542.

MACHOL, L. (1980). Breast-feeding revisited—The good news and some bad news. *Contemp. Ob/Gyn* **16,** 87–97.

MEHENDALE, H. B., AND MATTHEWS, H. B. (1973). Metabolism, excretion and storage of hexachlorobenzene in the male rate. In *Abstracts. American Chemical Society Meeting, Dallas, April 1973.*

MENDOZA, C. E., COLLINS, B. T., SHIELDS, J. B., AND LAVER, G. W. (1978). Effects of hexachlorobenzene or hexabromobenzene on body and organ weights of preweanling rats after a reciprocal transfer between the treated and control dams. *J. Agric. Food Chem.* **26,** 941–945.

MORITA, M., AND OISHI, S. (1975). Clearance and tissue distribution of hexachlorobenzene in rats. *Bull. Environ. Contam. Toxicol.* **14,** 313–318.

MORSE, R. W. (1975). *Assessing Potential Water Pollutants.* Natl. Acad. Sci., Washington, D.C.

MULL, R. L., WINTERLIN, W. L., PEOPLES, S. A., AND OCAMPO, L. (1978). Hexachlorobenzene. 1. Uptake, distribution and excretion of hexachlorobenzene (HCB) in growing lambs. *J. Environ. Pathol. Toxicol.* **1,** 865–878.

PETERS, H. A., CRIPPS, D. J., AND GOCMEN, A. (1978). Porphyria 20 years after hexachlorobenzene exposure. *Neurology* **28,** 333.

PETERS, H. A., JOHNSON, S. A. M., CAM, S., ORAL, S., MUFTU, Y., AND ERGENE, T. (1966). Hexachlorobenzene-induced porphyria: Effect of chelation on the disease, prophyrin and metal metabolism. *Amer. J. Med. Sci.* **251,** 314–322.

QUINLIVAN, S. C., AND CHASSEMI, M. (1977). Sources, characteristics and treatment and disposal of industrial wastes containing hexachlorobenzene. *J. Hazardous Mater.* **1,** 343–359.

RICHTER, E., RENNER, G., BAYERL, J., AND WICK, M. (1981). Differences in the biotransformation of hexachlorobenzene (HCB) in male and female rats. *Chemosphere* **10,** 779–785.

ROZMAN, K., MUELLER, W., IATROPOULUS, M., COULSTON, F., AND KORTE, F. (1975). Ausscheidung Koerperverteilung und Metabolisierung von hexachlorobenzol nach oraler Einzeldosis in Ratten und Rhesussaffen. *Chemosphere* **5,** 289–298.

SHIRAI, T., MIYATA, K., NAKANISHI, G., MURASAKI, G., AND ITO, N. (1978). Hepatocarcinogenicity of polychlorinated terphenyl (PCT) in ICR mice and its enhancement by hexachlorobenzene (HCB). *Cancer Lett.* **4,** 271–275.

STONARD, M. D. (1975). Mixed type hepatic microsomal enzyme induction by hexachlorobenzene. *Biochem. Pharmacol.* **24,** 1959–1963.

STONARD, M. D., AND NENOV, P. Z. (1974). Effect of hexachlorobenzene on hepatic microsomal enzymes in the rat. *Biochem. Pharmacol.* **23,** 2175–2183.

VILLENEUVE, D. C. (1975). The effect of food restriction on the redistribution of hexachlorobenzene in the rat. *Toxicol. Appl. Pharmacol.* **31,** 313–319.

VILLENEUVE, D. C., PHILLIPS, W. E. J., PANOPIO, L. G., MENDOZA, C. E., HATINA, G. V., AND GRANT, D. L. (1974). The effects of phenobarbital and carbon tetrachloride on the rate of decline of body burdens of hexachlorobenzene in the rat. *Arch. Environ. Contam. Toxicol.* **2,** 243–252.

VILLENEUVE, D. C., VAN LOGTEN, M. J., DEN TONKELAAR, E. M., GREVE, P. A., VOS, J. G., SPEIJERS, G. J. A., AND VAN ESCH, G. J. (1977). Effect of food deprivation on low level hexachlorobenzene exposure in rats. *Sci. Total Environ.* **8,** 179–186.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.